

PHENOTYPING BELGIAN BLUE CATTLE FOR THEIR SUSCEPTIBILITY TO PSOROPTIC MANGE

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INTRODUCTION

Psoroptic mange is a severe dermatitis caused by a mite infection with *Psoroptes ovis*. The lower weight gain, inferior leather quality and treatment costs cause economic losses (Lonneux et al., 1998). Moreover, the animals suffer from itch, crusts and wounds, leading to decreased animal welfare.

Belgian Blue cattle are extremely sensitive to this disease (Losson et al., 1999). The breed represents 51% of all cattle in Belgium, corresponding to 1.3 million Belgian Blue animals. A recent questionnaire (Sarre et al., 2012) demonstrates that 75% of all beef farms in Flanders are confronted with mange. The strong breed predisposition is an indication of contributing genetic factors in the development of psoroptic mange. Therefore a genome wide association study with the 50K bovine SNP-panel will be performed, which first requires a clear phenotype definition. It is the aim of this study to establish an objective phenotype allowing to differentiate mange susceptible and more resistant animals based on measurable clinical and parasitological parameters. The resulting definition can also be applied to epidemiological studies as a standard to facilitate more efficient sampling in wider geographical areas, since the method is more objective. In addition, a standardized phenotype might allow to combine smaller datasets in a meta-analysis.

MATERIAL AND METHODS

Animals

270 Belgian Blue cattle were sampled on 8 farms between February 2012 and April 2013. These farms are all part of the genomic project run by the Association Wallonne de l'Elevage (AWE). All animals were under 24 months of age. To identify the animal, eartag number, sex, color, stable/box and date of the visit were recorded along with the phenotypic parameters.

Timing farm visits

Every farm was visited at least 3 times. The visits were scheduled in the winter when animals are housed indoors and the mange prevalence is highest.

The first visit took place as soon as a mange outbreak was reported by the farmer. Two weeks later, the evolution of the disease was evaluated at the second visit and soon thereafter the animals were treated against mange. One month after treatment, the farm was visited a third time and a fourth visit was planned at the end of the housing period (April). If the third visit was too late in the season, no fourth visit was performed. The samples collected at these 3 or 4 visits are described in Table 1.

Table 1 Collection of clinical and parasitological parameters for the estimation of the mange phenotype by sampling at 4 visits in 1 winter

	Visit 1	Visit 2	Visit 3	Visit 4
Calculation of clinical index				
Scoring lesion appearance				
Mite counting				

Visit 1: as soon as possible after disease outbreak;

Visit 2: 14 days untreated; Visit 3: 1 month after (first) treatment;

Visit 4: end of winter

Sampling

The mange phenotype was defined based on lesion extent, lesion appearance and mite counts. The lesion extent or 'clinical index' is calculated as the affected proportion of the body surface. Therefore, the lesions are drawn on a sketch of both sides of the animal, divided in 350 squares (Figure 2, Guillot, 1981). The second parameter is the 'lesion appearance', recorded at every visit and classified as active, healing or healed lesions. Finally, *Psoroptes ovis* mites were counted in skin scrapings of each animal to establish the third parameter. Three skin scrapings of 4 cm² are taken at the edge of the lesions or if none, at the predilection sites of *P. ovis* mites, i.e. the withers, the centre of the back and the tail basis. The final mite score is a class variable between 0 and 3 with class separation at 1, 11 and 51 mites. Apart from *P. ovis* mites, the presence of other ectoparasites like *Chorioptes* or lice is also noted. At the third visit, no skin scrapings are collected because of a confounding effect of the acaricide treatment. Besides these phenotypic parameters blood samples for further genetic and immunologic research are collected at the first visit.

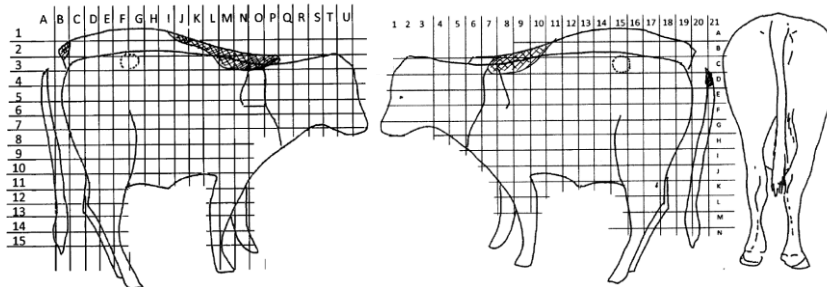


Figure 1. drawing the lesions on a grid to calculate the clinical index (adapted from Guillot, 1981)

RESULTS

The clinical index is considered to be the major contributor to the phenotype but also the lesion appearance and the mite score influence the overall disease assessment. Every parameter is arbitrarily weighed in accordance to its influence on the final phenotype. Before the three parameters are combined, they are centered and scaled as follows. Per visit en per farm the difference between the individual score en the mean score is divided by the standard deviation on that score (Figure 2). These centered scores are then combined in a "visit score" by summing these scores after weighing. The weighing was performed according to a Delphi method (consensus between experts into the consortium). The current model uses a linear equation in which 80% of the score is determined by the clinical index while both lesion appearance and mite count get 10% of the weight. Other equations with

different contributions of each parameter were found to be less correlated with subjective appearance. The visit scores of each individual animal are finally summed and sorted to result in a list with the extremely sensitive/resistant animals at each end. When records of a visit are missing, the average score on that farm for that visit is used as imputation method.

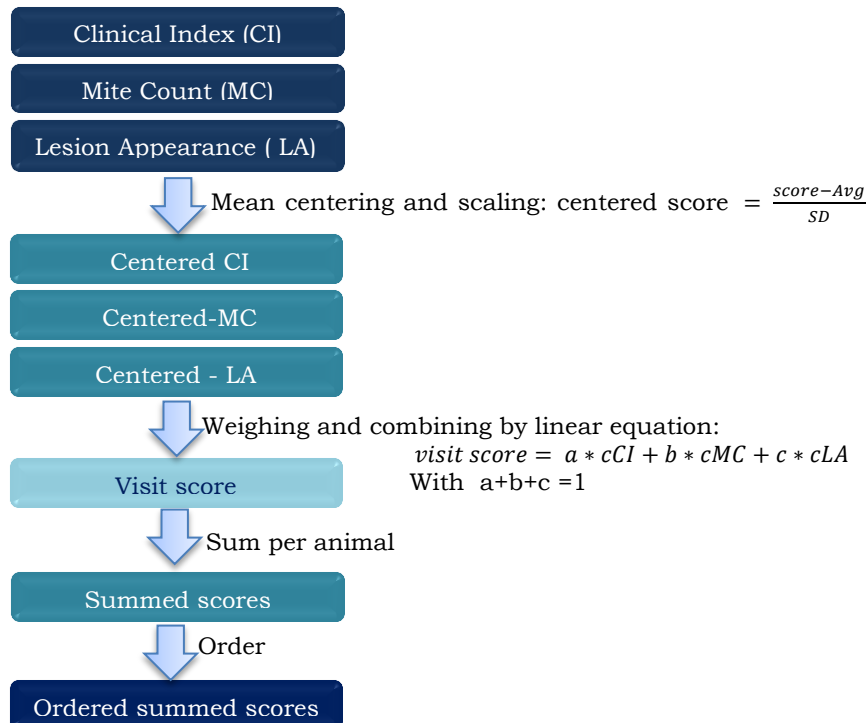


Figure 2. Schematic representation of the phenotyping procedure for the sensitivity to psoroptic mange in cattle

Using this formula, no clear threshold value could be established for mange susceptibility. As such, the sensitivity for psoroptic mange is considered to be a continuous variable, and the data will thus be treated in that way in the genetic analysis. Although the treatment depends on the individual farmer and cannot be controlled by the research team, the third visit has an added value: There was a trend with a positive R^2 of 0.06 for the correlation between the clinical index at the third and first visit and an R^2 of 0.17 at the third and second visit. However, this trend is not conclusive (R^2 too low), so information from the third visit cannot be predicted from the 2 former visits.

DISCUSSION

Although psoroptic mange in cattle was already studied in the 80s (Guillot, 1981; Stromberg et al., 1986), no general phenotyping definition was established. In order to enable the use of state-of-the-art genetic methodologies, a sufficiently large number of animals must be sampled and phenotyped with the same standard procedure. Traditional studies follow a few animals for at least 5 weeks and hence are not feasible for the intended 1000 animals necessary for the genomic analysis. Mite counts and different definitions of clinical scores were sometimes completed with the assessment of appetite (El-Khodery et al., 2009), body condition (Parker et al., 1999) or behaviour (Burgess et al., 2012). However, until now, no clear objective phenotype definition was established. The method presented in this paper facilitates the collection of many, geographically scattered, phenotypes in a standardised way.

The current model uses a linear equation in which the parameters are arbitrarily weighed by elicitation of experts. Further optimisation of this procedure might lead to an alternative higher order model. Using the current model, the mange phenotype is considered as a continuous trait. It should be noted that this continuous variable is partly created artificially by the methodology of phenotyping using clinical index or lesion area as primary variable. Analysing raw data as a continuous trait rather than 2 classes (susceptible or resistant), avoids the loss of valuable information of animals that cannot be clearly allocated to one class. This increases the power of the genome wide association study.

The unique feature of the presented procedure is the combination of 11 measurements on each animal, spread over 4 farm visits. Correlations between the visits showed the necessity of at least 3 visits for a correct assessment of the disease. However, the number of visits is still the main drawback: especially when few animals are housed on each farm, they are time consuming.

CONCLUSION

The phenotyping definition presented in this paper allows for systematic data collection. Using this model, the psoroptic mange phenotype is not considered as a class variable (sensitive or not) but rather as a continuous variable. This finding is of major value for the future genetic analysis that is planned.

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